comprising a variable region such that each member of the set hybridizes to a member of the array of probes; and

determining molecular weights of nucleic acids in the target array to identify hybridized probes, whereby the sequence of the target nucleic acid is determined.

REMARKS

A check for the fees for a Notice of Appeal and for a 3-month extension of time accompany this response. Any fee that may be due in connection with this application, including a fee for an extension of time, may be charged to Deposit Account No. 50-1213. If a Petition for extension of time is needed, this paper is to be considered such Petition.

Claims 1-55, 58-60, 63-76, 86, 88-125, 127, and 128 are pending in this application. Claim 1 is amended in order to more particularly point out and distinctly claim the subject matter. It is amended to more clearly define aspects of positional sequencing by hybridization. Basis for the amendment of claim 1 can be found throughout the specification as originally filed. For example, particular basis can be found at page 26, which recites:

> the typical probe array will comprise a collection of probes with sufficient sequence diversity in the variable regions to hybridize, with complete or nearly complete discrimination, all of the target sequence or the target-derived sequences. The resulting target array will comprise the entire target sequence on strands of hybridized probes.

THE REJECTION OF CLAIMS 1-27, 29-55, 58-60, 63-70, 73-76, 86, 88-125, 127, AND 128 UNDER 35 U.S.C. §102(e)

Claims 1-27, 29-55, 58-60, 63-70, 73-76, 86, 88-125, 127, and 128 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Köster (U.S. Patent No. 5,605,798) because Köster allegedly discloses processes for sequencing nucleic acids using mass spectrometry that disclose all elements of the claimed subject matter. This rejection is respectfully traversed.

Relevant Law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Spada*, 15 USPQ2d 1655 (Fed. Cir., 1990); *In re Bond*, 15 USPQ 1566 (Fed. Cir. 1990); *Soundscriber Corp. v. U.S.*, 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl. 1966). See, also, *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir.), *cert. denied*, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." *In re Lang*, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on the examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. *Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co.*, 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference. *In re Oelrich*, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

The claims

The claims are directed to methods for ***sequencing*** a target nucleic acid by hybridizing the target (or complement thereof) to an array of probes and determining the sequence by a sequencing by hybridization method, in which hybrids are identified by determining the molecular weight of members of the array to thereby determine the sequence of the target. In particular, the methods are directed to a sequencing by hybridization technique where a set of nucleic acid fragments, from a target nucleic acid, are hybridized to an array of nucleic acid probes to form a target array. Hybrids are detected by determining the molecular weights of members of the target array to thereby determine the sequence.

In particular, claim 1 as-amended recites:

> providing a set of nucleic acid fragments each containing a sequence that corresponds to a sequence of the target nucleic acid;
>
> hybridizing the set to an array of nucleic acid probes to form a target array of nucleic acids, wherein each probe comprises a single-stranded portion comprising a variable region such that each member of the set hybridizes to a member of the array of probes; and
>
> determining molecular weights of nucleic acids in the target array to identify hybridized probes, whereby the sequence of the target nucleic acid is determined.

Differences between the disclosure of Köster *et al.* and the claimed subject matter

Köster is directed to methods of detecting nucleic acids using mass spectrometry to determine a molecular weight. There are a variety of different embodiments in which nucleic acids in a sample are detected (*i.e.*, determining that it is in a sample). None of the methods of detection in Köster involve sequencing a target nucleic acid molecule. For example, Köster, in one embodiment, discloses attaching a capture sequence (C) to a support (SS). Köster at Fig. 1A. The capture sequence is chosen to specifically hybridize with a complementary sequence (*i.e.*, target capture site, TCS) on a target nucleic acid molecule (T). Köster at Fig. 1A and col. 4, lines 62-64. The target nucleic acid molecule (T) also contains a target detection site (TDS). Hence the target is displayed. The presence of the target detection site (TDS) is determined by hybridizing a detector (probe) nucleic acid sequence (D) to the target detection site (TDS) and detecting the detector by mass spectrometry. The method does not result in the sequence of a target nucleic acid, but rather determines whether a particular target is present in a nucleic acid by virtue of hybridization of a detector (not the target) to displayed targets. The sequence of a target is not determined.

Furthermore, in contrast to the instant methods in which an array of probes is provided, in the solid state format of the method of Köster, target molecules are presented (they are captured on a support), hybridized to a probe, and the presence of a particular target is detected by detecting a particular probe. Sequencing is not involved.

The word "sequencing" does not appear in Köster and Köster does not discloses any methods for sequencing nucleic acids. Köster does not disclose any methods that involve sequencing by hybridization in which a set of target molecules is hybridized to a set of probes, nor such a method in which hybrids are detected by determining their molecular weight so that the sequence of the target can be constructed by identifying the hybridized probes.

There are no methods in Koster in which an array of probes that contain a single-stranded portion containing a variable region is hybridized to a set of nucleic acid molecules that each contain a sequence that corresponds to a sequence in the target nucleic acid to produce a target array of probes and hybridized probes. Köster contains no reference to methods of sequencing, and instead only describes methods of detecting a target nucleic acid. See, *e.g.*, Köster at column 3, lines 51-53 and 60; column 5, lines 19-21 and 43-45; column 7, lines 19-21; column 10, lines 47-52; and column 11, lines 46 and 49-51 and 57-59.

For example, Köster states that "**[d]etection** of hybridization and the molecular weights of the captured target sequences provide information on whether and where in a gene a mutation is present" (Köster at column 12, lines 24-26). Also, claim 1 of Köster states that the claimed subject matter is "[a] process for detecting a target nucleic acid sequence" and not a method of sequencing. In the method of claim 1 of Köster, a detector (probe) is detected; its presence is indicative of the presence of the target. The sequence of the target is not determined.

The Examiner asserts that sequencing is inherently present in the method disclosed in Köster, which "clearly teaches sequencing of each nucleotide

present in the target molecule." In particular, the Examiner concludes that each individual nucleotide of a target nucleotide detection can be used for sequencing the whole target nucleic acid. Office Action at page 13. Köster does not disclose or suggest a method in which each individual nucleotide is identified and does not disclose sequencing each nucleotide in a target. In methods in which a nucleotide is present, a primer is hybridized to a target and is extended by one nucleotide if a mutation is present (or absent depending upon how the experiment is performed). If the primer gets extended it has one molecular weight, if it does not (*i.e.*, the mutation is not present), it has a different molecular weight. By virtue of the detected molecular weight, the presence of a particular nucleotide in a target can be inferred. In such methods the presence of a mutation is detected by determining what base is extended onto a primer. Such method requires an *a priori* knowledge of the sequence of the target or a portion thereof. In addition, differences between sequencing and detection make the methods taught by Köster inapplicable to sequencing applications. Köster states that "the process of this invention makes use of ***known sequence information*** of the target sequence and ***known mutation sites***." Köster at column 12, lines 14-16.

Further, Köster does ***not*** disclose an array of nucleic acid probes, where each probe has a single-stranded portion having a variable region, for hybridizing to a set of nucleic acid fragments from a single target nucleic acid, as suggested by the Examiner. In making the rejection, the Examiner cites to Example 1; claim 1; Figure 1; column 4, lines 11-14; column 9, lines 28-43; and Figs. 2-3 for support. None of these sections, nor Köster as a whole, provide the support suggested by the Examiner. For instance, Example 1 is directed to an embodiment where a 50 nucleotide sequence (50-mer) attached to controlled pore glass beads serves as a template for separate hybridizations with a 26-mer ***or*** a 46-mer. Köster at column 12, line 53. Oligonucleotide not bound to the polymer-bound template is removed by centrifugation and washing, and the beads are mixed with matrix and analyzed by MALDI-TOF mass spectrometry.

If, arguendo, the 50-mer attached to the glass beads is construed to be a "probe" as used in the instant application, there is ***no*** variable region because the same 50-mer is attached to each of the controlled pore glass beads, and hence the sequence is identical. Alternatively, if the 26-mer or the 46-mer were considered to be the "probe," again there is no variable region, as the sequence of both the 26-mer and the 46-mer remains unvaried. Furthermore, Example 1 was provided to show that it is possible to capture a detector nucleic acid molecule on a solid support which is presenting a target molecule, and then detecting the hybridized detector by mass spectrometry.

Claim 1 also does not disclose an array of nucleic acid probes. Claim 1 recites:

> 1. A process for detecting a target nucleic acid sequence present in a biological sample, comprising the steps of:
> a) obtaining a nucleic acid molecule containing a target nucleic acid sequence from a biological sample;
> b) hybridizing a detector oligonucleotide with the target nucleic acid sequence, wherein at least one of the detector oligonucleotide or the target nucleic acid sequence has been conditioned;
> c) removing unhybridized detector oligonucleotide;
> d) ionizing and volatizing the product of step c); and
> e) detecting the detector oligonucleotide by mass spectrometry, wherein
> detection of the detector oligonucleotide indicates the presence of the target nucleic acid sequence in the biological sample.

Claim 1 is directed to an embodiment of a process for detecting a target nucleic acid that includes the steps of obtaining nucleic acid molecule containing a target nucleic acid sequence, hybridizing a detector oligonucleotide to the target, and then detecting the detector, where detection of the detector indicates that the target is present in the sample. Even assuming arguendo that the detector oligonucleotide of Köster is equivalent to the instantly claimed "probe," claim 1 of Köster makes no mention of arrays of probes; in the solid state methods of Köster, the target is displayed on a solid surface, not the

probes. In particular, claim 1 of Köster makes no mention of arrays with 4^R probes as in instant claim 124 and its dependents.

Figure 1 also does not disclose an array of nucleic acid probes. Figure 1 shows a process for performing mass spectrometry analysis on a target detection site (TDS) contained within a target nucleic acid molecule (T). A specific capture sequence (C) (Figs. 1A and 1C) or the target containing a detection site (Fig. 1B) is attached to a solid support (SS) via a spacer (S). The capture sequence (C) hybridizes with a complementary sequence on the target nucleic acid molecule. Hybridization between the detector nucleic acid sequence and the detector site can be detected by mass spectroscopy. None of Figs. 1A through 1C shows an "array of nucleic acid probes," but instead show only a single oligonucleotide attached to a solid support. Further, none of Figs. 1A-C show a probe containing a single-stranded portion having a variable region. None of these figures disclose a method in which the sequence of a target is deduced based upon probes to which it hybridizes.

The specification at column 4, lines 11-14; column 9, lines 28-43; and Fig. 2 also do not teach an array of nucleic acid probes. Each of these sections relate to "multiplexing." The Examiner alleges that this "multiplexing" discloses an array of probes each of which contains a single-stranded portion containing a variable region. As used in Köster at columns 4 and 9, and Fig. 2, multiplexing involves detection of a plurality of different ***target nucleic acid sequences*** in a single sample. Multiplexing does ***not*** relate to the use of a plurality of different ***probes***, as contained in the present application, but rather detection of a plurality of different target molecules.

In addition, although Fig. 3 illustrates a series of probes, each containing a different capture sequence, the probes are utilized to accomplish differentiation between ***multiple*** target nucleic acids (column 5, lines 52-62). Accordingly, the probes in Fig. 3 of Köster do not hybridize to a set of nucleic acid fragments from a single target nucleic acid, as required by the pending claims.

In addition, since Köster is concerned with detection and not sequencing, Köster does not describe determining the sequence of the target nucleic acid from molecular weights for nucleic acids of the target array. In making the rejection, the Examiner refers to Example 1, Claim 1, and Figs. 1-11 of Köster. Example 1, claim 1, and Figs. 1-3 are discussed above and do not disclose a target array (*i.e.*, a set of nucleic acid fragments from a single target nucleic acid hybridized to an array of nucleic acid probes); the array of nucleic acids comprise the target, not the probe (detector). Also, Fig. 4 shows a format where a ***predesigned*** target capture sequence is incorporated into the target sequence using PCR amplification. Fig. 5 relates to detection of amplification products by mass spectrometry. Fig. 6A describes mass spectrometric analysis of an amplified nucleic acid. Figs. 6B and C relate to mutliplexing. Fig. 7 describes a format detecting both strands of a target DNA. Fig. 8 relates to methods for determining whether and where mutations in a gene are present. Figs. 9 and 10 relate to Example 1 (described above). Fig. 11 relates to differentiation of an 18-mer and 19-mer (Example 2). Accordingly, none of the sections referred to by the Examiner, and indeed Köster when taken as a whole, discloses determining the sequence of the target nucleic acid from molecular weights for nucleic acids of the target array.

Köster does not disclose a method in which a set of nucleic acid fragments each containing a sequence that corresponds to a sequence in the target nucleic acid is provided, nor a method in which the set is hybridized to an array of nucleic acid probes to form a target array of nucleic acids and then determining molecular weights for nucleic acids of the target array to then determine the sequence of the target nucleic acid. Therefore, since anticipation requires disclosure in single reference of all elements as claimed, Köster does not anticipate any of the pending claims.

THE REJECTIONS OF CLAIMS 28, 71 and 72 UNDER 35 U.S.C. §103(a)

Claim 28

Claim 28 is rejected under 35 U.S.C. §103(a) over Köster (U.S. Patent No. 5,605,798) in view of Weiss (U.S. Patent No. 6,025,193) because Köster allegedly teaches all elements of claim 28, except generation of thiol moieties by using Beucage reagent, but the Examiner alleges that Weiss cures this defect. Applicants respectfully traverse the rejection.

Relevant Law

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. §103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (*ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. App. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" (*In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981)), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher." *W.L. Gore & Associates, Inc. v. Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

Under 35 U.S.C. §103, in order to set forth a case of *prima facie* obviousness, the differences between the teachings in the cited reference must

be evaluated in terms of the whole invention, and the prior art must provide a teaching or suggestion to the person of ordinary skill in the art to have made the changes that would produce the claimed product. *See, e.g., Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co.*, 730 F.2d 1452, 1462, 221 U.S.P.Q.2d 481, 488 (Fed. Cir. 1984). The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); *In re Papesh*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963).

Analysis

The Examiner has failed to set forth a *prima facie* case of obviousness.

There would have been no motivation to have combined the teachings of Weiss with those of Köster

There would have been no motivation to one of ordinary skill in the art to have combined Köster and Weiss in the manner suggested by the Examiner. Weiss teaches methods and compositions for diagnosing and treating pathological conditions related to a dopamine receptor abnormality. The reference teaches that unmodified oligodeoxynucleotides can be converted into phosphorothioate oligodeoxynucleotides using standard phosphoramidite protocols but replacing the standard oxidation by iodine with Beucage reagent for sulfurization. Weiss teaches that using Beucage reagent results in the replacement of every oxygen group of the phosphodiester bond with a sulfur group, and that such substitutions result in an asymmetric distribution of the negative charge to predominate on the sulfur atom, resulting in "improved stability to nucleases, retention of solubility in water and stability to base-catalyzed hydrolysis" (column 13, lines 2-14), improved biodistribution and *in vivo* stability (column 15, lines 41-45), and activation of Rnase H, and thus are potentially useful therapeutic agents (column 13, lines 45-47). Since Weiss is not concerned with methods for detecting or sequencing nucleic acids it's teachings are unrelated to the methods of Köster. Accordingly, those of

ordinary skill in the art would not have been motivated to have combined the teachings of the references. The advantages of using Beaucage reagent articulated by Weiss are inapplicable to detection or sequencing methods.

Further, Weiss does not teach or suggest the methods of sequencing by determining molecular weights of nucleic acids. Weiss also does not teach an array of nucleic acid probes each of which includes a single-stranded portion and a double-stranded portion. In addition, the reference does not teach or suggest a method for detecting or determining the sequence of the target nucleic acid by determining the molecular weights for nucleic acids of such an array.

The combination of teachings of Köster and Weiss does not result in the instantly claimed methods

Notwithstanding the lack of motivation to have combined the teachings of Köster and Weiss, such combination does not result in the instantly claimed methods. Specifically, as discussed above, Köster does not disclose methods for sequencing a target nucleic acid molecule. In particular, Köster does not teach a method that includes any of the steps of providing a set of nucleic acid fragments each containing a sequence that corresponds to a sequence in the target nucleic acid, hybridizing the set to an array of nucleic acid probes to form a target array of nucleic acids, and then determining molecular weights for nucleic acids of the target array to then determine the sequence of the target nucleic acid. Even if Weiss teaches generation of thiol moieties using Beaucage reagent, it fails to cure the deficiencies in the teachings of Köster. Therefore, the combination of teachings of Köster and Weiss does not result in any of the instantly claimed methods.

Claims 71 and 72

Claims 71 and 72 are rejected under 35 U.S.C. §103(a) as being unpatentable over Köster (U.S. Patent No. 5,605,798) in view of Sanghvi *et al.* (U.S. Patent No. 6,214,551) because Köster allegedly teaches all elements of the claims except that the selectively releasable bond is 4,4'-dimethoxytrityl or a

derivative thereof, and Sanghvi *et al.* allegedly cures this defect. The Examiner contends that Sanghvi *et al.* teaches the selectively releasable bond 4,4'-dimethoxytrityl or a derivative thereof, and argues that although the reference does not teach the derivative 3 or 4 [bis-(4-methoxy-phenyl)]-methyl-benzoic acid in particular, Sanghvi *et al.* teaches equivalent compounds and derivatives used for the same purpose. In making the rejection, the Examiner alleges that our previous argument was unpersuasive because the references were individually attacked instead of addressing the combination of the references. This rejection is respectfully traversed.

Relevant Law

See above in connection with the rejection of Claim 28.

Previous response

First it is noted the previous response did address the combination of the teachings of the references and did not "attack" them individually. Attention is directed to the section at page 20 of the previous response with the header "ANALYSIS" and "**The combination of cited references does not result in the instantly claimed methods**", which states in part:

> The combination of the teachings of Köster with Sanghvi *et al.* does not result in the subject matter of the pending claims. As discussed above (see page 8), Köster does not teach a method of <u>sequencing</u> a target nucleic acid, and Sanghvi *et al.* does not cure this defect because Sanghvi *et al.* does not teach or suggest a method for sequencing a target nucleic acid. Thus, neither Köster nor Sanghvi *et al.*, singly or in combination, teaches sequencing a target nucleic acid, and therefore the combination of Köster and Sanghvi *et al.* fails to teach all the elements of the subject matter of claims 1-27, 29-55, 58-60, 63-70, 73-76 of the instant application.

Analysis

As discussed above, Köster does not teach an array of probes or processes for determining the sequence of a target nucleic acid. Accordingly, even if the disclosure in Sanghvi *et al.* could be used to teach selectively releasable bonds containing 4,4'-dimethoxytrityl or a derivative thereof in the

processes described by Köster, the combination of Köster and Sanghvi *et al.* would not teach or suggest all of the features of the claimed methods.

In addition, Sanghvi *et al.* teaches the use of dimethoxy-trityl groups only as a blocking group during nucleoside polymerization. The reference does ***not*** teach or suggest the use of dimethoxytrityl or a derivative thereof as a selectively releasable bond by which to attach a probe to a solid support, as claimed in the instant application. Accordingly, Sanghvi *et al.* does not teach or suggest the element of the instantly claimed subject matter missing from Köster. Thus, combining the teachings of Sanghvi *et al.* with Köster does not result in the claimed subject matter.

CONCLUSION

Thus, none of the references, singly or in any combination, teaches or suggests a method for sequencing a target nucleic acid molecule by mass spectrometric analysis. Instead, the Examiner continues to conduct an improper "hindsight" analysis in which he picks and chooses the elements of "sequencing," "target nucleic acid," "Beucage reagent," and "dimethoxytrityl" from the various references to combine them as claimed in the instant application.

JOINT INVENTORS (102(f) AND 102(g))

The instant application, which is a continuation of U.S. patent application Serial Nos. 08/420,009; 08/470,835; 08/419,994; and 08/470,716, designates as joint inventors: Charles R. Cantor and Hubert Köster, each of whom was subject to an obligation to assign to a different entity. Applicant is aware of the obligation imposed by 37 C.F.R. §1.56. Upon investigation, it is believed that both named inventors are joint inventors for each of the currently pending claims. If the Office believes that a rejection of any claims based upon 35 U.S.C. §102(f) and/or 102(g) can be made if claims have different inventors, the Office is invited to do so, so that applicant can then investigate actual inventorship of the claims at issue and/or ascertain a date of invention.

* * *

In view of the above amendments, consideration and allowance of the application are respectfully requested.

Respectfully submitted,
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Jonathan Ong

MARKED UP CLAIMS IN ACCORDANCE WITH 37 C.F.R. § 1.121

1. A method for sequencing a target nucleic acid, comprising the steps of:

providing a set of nucleic acid fragments each containing a sequence that corresponds to a sequence of the target nucleic acid;

hybridizing the set to an array of nucleic acid probes to form a target array of nucleic acids, wherein each probe comprises a single-stranded portion comprising a variable region[,] such that each member of the set hybridizes to a member of the array of probes; and

determining molecular weights [for] of nucleic acids [of] in the target array to identify hybridized probes,[;] whereby the sequence of the target nucleic acid is determined.